

TECHNICAL NOTE

Variable specificity of Tn7::*lacZY* insertion into the chromosome of root-colonizing *Pseudomonas putida* strains

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Tn7 is unique among prokaryotic transposons in that it typically transposes at high frequency to a specific target site, *attTn7*, located near the *glmS* gene in *E. coli* (Craig 1989; Waddell & Craig 1988) and in various pseudomonad species (Caruso & Shapiro 1982; Boucher *et al.* 1985; Barry 1986). It also exhibits transposition immunity, i.e. the presence of a Tn7 insertion greatly reduces the frequency of a second insertion in the same DNA molecule (Hauer & Shapiro 1984; Arciszewska *et al.* 1989). These properties make Tn7 especially suitable for single, site-specific insertion of marker genes into the bacterial chromosome. In particular, the monocomponent Tn7::*lacZY* system, based on the donor plasmid, pMON7197, carrying *lacZY* between the arms of Tn7, as well the transposition genes, *tnsABCDE* (Barry 1988), has been shown to produce pseudomonad isolates that had *lacZY* permanently inserted into their chromosomes, expressed β -galactosidase at high levels and exhibited no alterations in their growth rates or nutritional requirements (Cook *et al.* 1991; Kluepfel *et al.* 1991; Parke *et al.* 1992; Hartel *et al.* 1994).

A number of *lacZY*-labelled pseudomonads, derived from the pMON7197-based system (Barry 1988), have been used in field studies. Kluepfel *et al.* (1991) used *Pseudomonas aureofaciens* Ps3732RN Lac⁺ in South Carolina, Parke *et al.* (1992) used *P. fluorescens* PRA25 Lac⁺ in Wisconsin, and Cook *et al.* (1991) used *P. fluorescens* 2-79RN Lac⁺, and *P. aureofaciens* Q2-87 Lac⁺ and QC-65 Lac⁺, in Washington. In addition, *P. corrugata* 2140R Lac⁺ has been released in Australia (M. Ryder 1994, ECSIRO, pers. comm.). In all of these well characterized or 'type' isolates, Southern analysis revealed that each had a single insertion in the chromosome.

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To our knowledge, only one report has appeared in the literature that specifically addressed the question of insertion specificity of Tn7 in pseudomonads. Barry (1986), in an examination of four exconjugants derived from *P. fluorescens* 701E1 with the use of the bicomponent (pMON7029 and pMON7018) system, found all of them to have a single *lacZY* insertion in the same location in the chromosome. We report here that broader application of this *lacZY* marking system to environmental pseudomonads has revealed that not all strains appear to share the feature of a single, preferred *lacZY* insertion site.

The parental (recipient) strains, *P. putida* GR12-2 (Lifshitz *et al.* 1986), *P. putida* R20 (Meyer & Linderman 1986), and *P. putida* PH6 (Fuhrmann & Wollum 1989), were isolated from the roots of various plant species. Recipient strains were cultured at 25 °C in *Pseudomonas* F medium (PF; Difco Laboratories) containing 1% glycerol (v/v). Genomic fingerprints (Drahos & Barry 1992) of *Eco*R1-digested DNA indicated that all three parental strains were distinctly different (data not shown).

The donor plasmid (pMON7197, 26.1 kb; Staley & Drahos 1994) was carried in *E. coli* MM294, while the helper plasmid (pRK2013, 48 kb) was carried in *E. coli* HB101 (ATCC 37159). Both the donor and helper strains were cultured at 37 °C in Luria-Bertani (LB) medium containing gentamicin (15 mg/L) or kanamycin (50 mg/L), respectively. Details of the triparental mating and selection procedure are given elsewhere (Staley & Drahos 1994). For each pseudomonad strain, exconjugants were isolated from at least three separate matings by picking blue colonies as they appeared at 30 °C on minimal lactose (D-lactose monohydrate; Difco Laboratories) and X-gal [5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (5 Prime \rightarrow 3 Prime, Inc.)] medium (MLX).

DNA isolation, digestion, blotting and hybridization were carried out using standard procedures (Maniatis *et al.* 1982). The gene probe was prepared from pMON7117 (11.2 kb) carried in *E. coli* MM294 (Barry 1988). Double digestion of the plasmid with *Bgl*III, followed by *Hind*III (both from United States Biochemical Corp.), produced three fragments of 7.0, 3.2 and 1.0 kb. The 7.0 fragment,

spanning the *lacZYA'* sequences (Barry 1988), was electroeluted and covalently linked via glutaraldehyde to peroxidase for use in the enhanced chemiluminescence procedure (ECL; Amersham Corp.). Specificity of the probe for the *lacZY* genes on the donor plasmid was confirmed via ECL, with pMON7197 either singly digested with *Eco*RI or doubly digested with *Eco*RI and *Bam*HI (United States Biochemical Corp.). The 7.0-kb probe from pMON7117 showed homology only for the 5.7- and 4.2-kb fragments, each containing about half of the *lacZYA'* sequences from *Eco*RI-digested pMON7197. Double digests with *Eco*RI and *Bam*HI yielded hybridizable fragments of 4.9 and 4.2 only, which is in accord with the removal of the 0.9-kb fragment between the *Eco*RI site and Tn7 L.

Southern analysis of *Eco*RI-digested, genomic DNA showed, as expected, that Tn7 had a high specificity of insertion of *lacZY*, and as a single copy, into the chromosome of all nine exconjugants obtained from strain GR12-2 (Fig. 1a). However, a number of the exconjugants within each of the series from the two other strains, R20 and PH6, had *lacZY* inserted at different locations (Fig. 1b and c, respectively). Of nine exconjugants examined from strain R20 (Fig. 1b), five had distinctly different insertion sites, while two had insertion sites identical to one other exconjugant. Of eight exconjugants examined from strain PH6 (Fig. 1c), nearly all appeared to have different *lacZY* insertion sites. As indicated above, single insertions were found in all of the exconjugants from strain GR12-2. Likewise, single insertions were found for all the exconjugants from strain R20 (Fig. 1b). Unexpectedly, exconjugants from strain PH6 apparently had from one to possibly four *lacZY* insertions in their chromosomes (Fig. 1c).

Within a series of exconjugants derived from any one strain, all retained the distinctive, genomic fingerprint of their parental strain (data not shown), thus confirming their isogenicity.

Three type isolates (LRO102 from *P. putida* GR12-2, LRO101 from *P. putida* R20, and LRO215 from *P. putida* PH6) were extensively characterized. With the exception of the hydrolysis of *p*-nitrophenyl- β -galactopyranoside (an analogue of lactose), no differences were found between the parental strain and its type isolate for 22 physiological tests (NFT kit), 12 antibiotic sensitivities, and growth rate in minimal glucose broth. All the other isolates from GR12-2 were similarly identical to the parental strain. In contrast, many of the other isolates from R20 and PH6 showed differences in growth rate and colony morphology on MLX agar plates, which is consistent with insertions at different sites.

Heretofore, it has been assumed that Tn7 transposes at high frequency to a specific target site, *att*Tn7, in the chromosome of pseudomonads. Of the work that has been published, only single insertions in the same location in

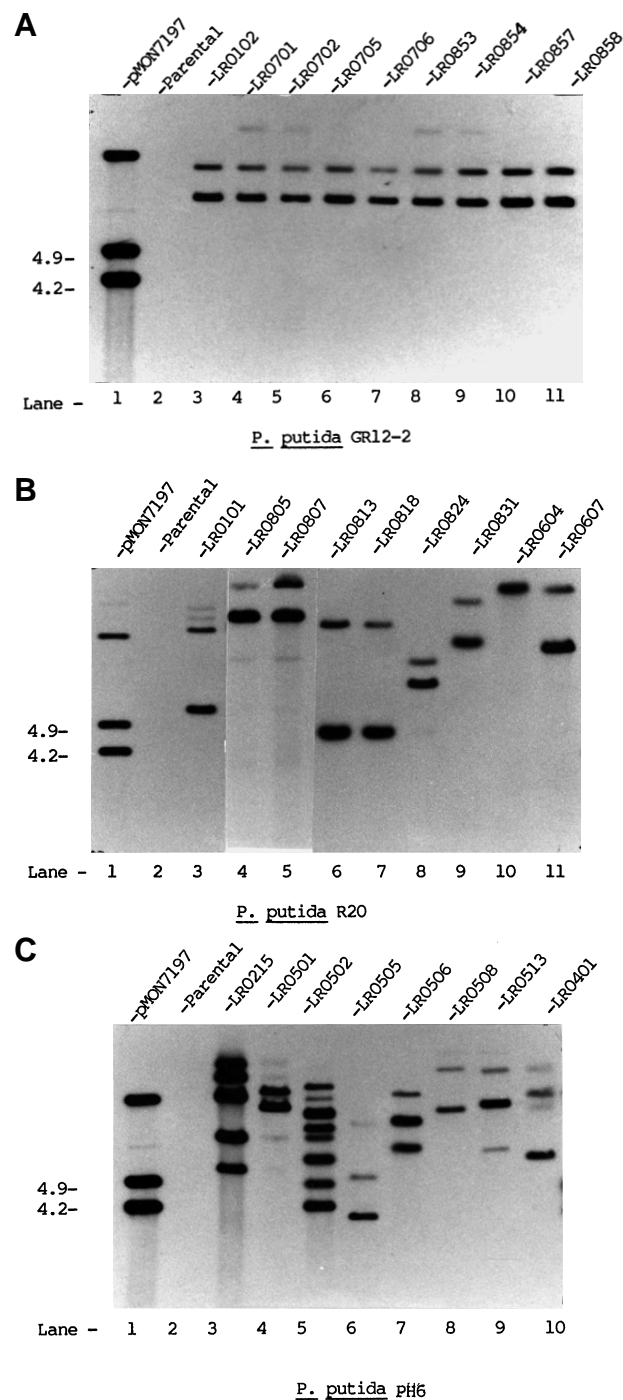


Fig. 1 Southern blot of *Eco*RI-digested genomic DNA from various pseudomonad parental strains, and their exconjugants, probed with 7.0-kb DNA fragment containing *lacZY* from pMON7117 (pMON7197 DNA was digested with *Eco*RI and *Bam*HI).

the chromosome of pseudomonads have been reported, i.e. putative *att*Tn7 sites were present. Tn7 transposition into the *E. coli* chromosome, which has been extensively studied, has been shown to be exclusively in *att*Tn7 sites, if present, and if at least *tnsABCD* are functional (Kubo &

Craig 1990; Rogers *et al.* 1986; Waddell & Craig 1988). Because the pMON7197-based, Tn7 transposition system provides, presumably, functional *tnsABCDE*, and *attTn7* sites are apparently common in pseudomonad species, it was logical to assume that we would also find high specificity of Tn7::lacZY in our *P. putida* strains. Although this assumption appears to hold for *P. putida* GR12-2, our results suggest that, at least in *P. putida* R20 and *P. putida* PH6, *attTn7* sites are either present in multiple copies or absent. Insertion in a strain without an *attTn7* site would occur at a less-preferred secondary site. Whether such secondary sites in the R20 and PH6 strains resemble either the pseudo-*attTn7* or other Tn7 secondary sites in *E. coli* (Craig 1989; Waddell & Craig 1988) has yet to be determined.

Our results also demonstrate that the use of Tn7::lacZY may not always result in innocuous insertions in *P. putida*, especially if only secondary sites are available in the chromosome. Therefore, thorough characterization (including molecular genetic) and careful selection of lacZY-marked pseudomonads derived using the pMON7197-based system are indicated, especially when isolates are to be used in environmental studies, to insure that the parental and marked strains are as identical as possible.

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